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In a particularly preferred embodiment, the nucleic acid sequence derived from an ASK-gene is an ASKdzethaASKdzeta or an ASKetha gene. ASK is the abbreviation for Arabidopsis SHAGGY-related protein kinases (Dornelas et al., 1998). The cDNA and genomic DNA sequences of various ASK-gene, including the ASK-genes of group II, are published in Dornelas et al. Gene 212 (1998), 249-257 and Dornelas et al. Plant Molecular Biology 39, (1999) 137-147, whose content with respect to the sequence and its provision is fully incorporated herein by reference. This article contains the reference to Accession X94938 in the GenBank. The Arabidopsis thaliana mRNA for shaggy-like Kinase Dzeta identified as Accession X94938 is provided herein as Seq. Id. No. 6. In the context of the present invention, ASK-genes of group II are the ASK genes classified according to Dornelas et al. (199) in group II of SGG/GSK-3 homologues, in particular ASKiota, ASKdzethaASKdzeta and ASKetha. In a particularly preferred embodiment, the ASK-genes of group II of the present invention are ASKdzethaASKdzeta and ASKetha genes.

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According to the present invention, the DNA constructs, in particular the antisense and sense constructs used, comprise a nucleic acid sequence derived from an ASK-gene of group II, in particular the ASKdzethaASKdzeta and/or ASKetha gene, or parts thereof.

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In a preferred embodiment of the present invention, the use of the ASKdzethaASKdzeta gene in antisense constructs or in sense constructs used from instance in co-suppression technology (co-suppression constructs) for eliminating wild-type seeds, whose embryos and seedlings are characterized by the development of, in contrast to that of a wild-type plant, an increased number of cotyledons obviously caused, without being limited by theory, by abnormal divisions of the hypophyseal cell and abnormal development of the upper and lower tiers of the embryo. As a consequence, the embryo and seedling exhibits supernumerary cells and shows polycotyly.

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In a further preferred embodiment, it is contemplated to use both ~~ASKdzetha~~ASKdzeta and ASKetha genes in the antisense or co-suppression construct of the present invention enabling the production of a transgenic plant the embryos of which are characterized by an abnormal development, in the course of which embryos containing both ~~ASKdzetha~~ASKdzeta and ASKetha antisense or co-suppression construct fail to form a distinct suspensor/embryo proper structure and abort, preferably already after a few cell divisions. Accordingly, the use of ~~ASKdzetha~~ASKdzeta and ASKetha genes together in an antisense or co-suppression construct enables the production of plants, the seeds of which are characterized by the abortion of the embryo as well.

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Figure 3 shows the cloning scheme for obtaining the ~~ASKdzetha~~ASKdzeta antisense construct.

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These fragments were cloned in antisense orientation under the strong constitutive CaMV 35S promoter, in a modified version of the *Agrobacterium tumefaciens* pEC plasmid (INRA, Versailles, France plasmid map, Fig. 3). Fig. 3 shows the cloning scheme indicating that the ~~ASK-dzetha~~ASKdzeta 5' region defined above is cloned in antisense orientation to the 35S CaMV promoter and is functionally linked to the 35S CaMV 3' transcription termination region (construct: ASK  $\alpha$  AS). The expression cassette obtained is cloned together with the bar gene between the left and right border sequence of *Agrobacterium tumefaciens*. At least 18 plants were obtained for each of the ASK antisense constructs. Transformation, cultivation and regeneration were carried out using standard protocols. Transformed plants were left to self-pollinate and the progeny was tested for the presence of the construct insertion by PCR using primers in the ASK genes in combination with the TAG 17 primer on the pEC T-

DNA (5'-GAGCCGCAGGAACCGCAGGAGTGCA-3', SEQ ID No. 5). The amount of native ASK (about 1,6 kbp) and antisense (about 0,3 kbp) transcript levels was accessed by Northern blot experiments, using ASK gene-specific probes under the conditions described in Dornelas et al., 1999.

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In order to assess the effect of the reduction of transcript levels of both ASK-genes simultaneously, more than 40 independent crosses were performed among homozygous ASK antisense plants. Embryos carrying both ASK antisense constructs were obtained by crossing homozygous ASK $\eta$  and – $\zeta$  antisense plants.

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Embryo development of ASK $\zeta$  $\eta$  antisense embryos showed abnormal development as early as the first divisions of the suspensor. The uppermost suspensor cell showed features comparable to the cells of the embryo proper cells such as having less vacuolated cytoplasm and similar cell shape. As the first longitudinal divisions in the apical cell took place to produce a guardant embryo proper, an abnormal, longitudinal cell division of the uppermost suspensor cell (the hypophyseal cell) occurred. In the wild-type embryos the uppermost suspensor cell exclusively undergoes transversal mitotic divisions and only at the early globular stage to form the hypophysis.

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At the guardant stage of the ASK $\zeta$  $\eta$  embryo development, the mitotic transversal divisions of the embryo proper cells proceeded. Simultaneously, the daughter cells resulting from the abnormal cell division of the hypophyseal cell mimicked the division patterns highly characteristic of the terminal embryonic cell. As a result of these aberrant division patterns, the embryo proper contained twice as many cells when compared to the wild-type. At the dermatogen state, the cells derived from the hypophyseal cell underwent periclinal divisions, giving

rise to protoderm-like cells from this stage onwards. The latter cells divided only anticlinally, behaving like protoderm cells. At the globular stage of the ASK $\zeta$ dzeta antisense embryo development, the second uppermost suspensor cell had undergone transverse division and formed a hypophysis-like structure. Altogether, these aberrant cell divisions resulted in an embryo showing an ovoidal rather than a globular shape.

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Both in the wild-type as in the ASK $\zeta$ dzeta antisense embryos, at the late globular to heart stage protodermal divisions increased in frequency at the site of the future cotyledons. These cell divisions resulted in a triangular shaped embryo. The cotyledon initials which were formed at the apical region of the ASK $\zeta$ dzeta antisense embryo were supernumerary in most cases (70% of the embryos analyzed,  $n > 100$ ). Thus, when cells that will form the cotyledons are recruited at the late globular stage of ASK $\zeta$ dzeta antisense embryos, as much as twice the amount of cells were available. Consequently, up to six cotyledons were detected in mature ASK $\zeta$ -ASKdzeta antisense embryos (Fig. 1). At the torpedo stage, the supernumerary cotyledons were visible in cleared seeds. With further elongation of the cotyledons and the bending of the embryo, the seeds of the ASK $\zeta$ dzeta antisense plants showed a roundish shape when compared to the wild-type, due to the accommodation of the supernumerary cotyledons.

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After germination of ASK $\zeta$ dzeta antisense seedlings displayed a normal shape, except that they showed an increased number of cotyledons (polycotyly) (Fig. 2). Ninety percent of the seedlings presenting polycotyly showed 3 cotyledons, while ten percent showed 4-6 cotyledons. In this latter case, cotyledons were reduced in size. The relative position of the first leaves, which alternate with the insertion of cotyledons, was maintained in the ASK $\zeta$ dzeta antisense plants.

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As described in example 2, an early developmental defect was also detected during the first mitotic divisions of the ASK $\eta$ etha antisense embryos. The uppermost suspensor cell of the latter embryo had less vacuolated cytoplasm and the shape of the embryo proper cells. After the first longitudinal division of the embryo proper cell, the hypophyseal cell divided abnormally (i.e. longitudinally) and the adjacent suspensor cell became less vacuolized. At the guardant stage, the daughter cell resulting from normal division of the hypophyseal cell occasionally divided again and the adjacent suspensor cell underwent an abnormal, longitudinal division.

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At the globular stage of the ASK $\eta$ etha antisense embryos, the embryo proper cell had not differentiated into typical protodermal cells as it is observed in the wild-type embryos at this stage. Instead further abnormal mitotic divisions of the suspensor proceeded towards the lower cells. AT the late-globular stage, the embryo proper cell divided irregularly and the suspensor cells divided further, causing the ASK $\eta$ etha antisense embryo to adopt a club-shaped form. At this stage, ASK $\eta$ etha antisense plants showed 70-100% seed abortion.

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Embryo Development of ASK $\eta$ etha and ASK $\zeta$ dzeta Antisense Plants

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In order to assess the effect of the reduction of both ASK $\zeta$ dzeta and ASK $\eta$ etha transcript levels simultaneously, homozygous ASK $\zeta$ dzeta and ASK $\eta$ etha antisense plants were crossed. Double ASK $\zeta$  $\eta$ dzeta/etha antisense embryos showed abnormal cell divisions, starting from the first division of the apical cell. The division planes of both basal and apical cells varied in a great extent. The ASK $\zeta$  $\eta$ dzeta/etha antisense embryos failed to develop further than the globular stage and the seeds aborted. The phenotype of the double ASK $\zeta$  $\eta$ dzeta/etha antisense embryo is thus more severe than the transcript levels of each

individual gene are reduced. This suggests that both genes may act in different pathways to transduce signals that are essential for the progression of Arabidopsis embryo development beyond the globular stage.